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X Chromosome Inactivation and X-Linked Mental Retardation

Huntington F. Willard

Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio

The expression of X-linked genes in females heterozygous for X-linked defects can be modulated by epigenetic control mechanisms that constitute the X chromosome inactivation pathway. At least four different effects have been found to influence, in females, the phenotypic expression of genes responsible for X-linked mental retardation (XLMR). First, non-random X inactivation, due either to stochastic or genetic factors, can result in tissues in which one cell type (for example, that in which the X chromosome carrying a mutant XLMR gene is active) dominates, instead of the normal mosaic cell population expected as a result of random X inactivation. Second, skewed inactivation of the normal X in individuals carrying a deletion of part of the X chromosome has been documented in a number of mentally retarded females. Third, functional disomy of X-linked genes that are expressed inappropriately due to the absence of X inactivation has been found in mentally retarded females with structurally abnormal X chromosomes that do not contain the X inactivation center. And fourth, dose-dependent overexpression of X-linked genes that normally "escape" X inactivation may account for the mental and developmental delay associated with increasing numbers of otherwise inactive X chromosomes in individuals with X chromosome aneuploidy.

KEY WORDS: X inactivation, aneuploidy, X chromosome, functional disomy, mental retardation

INTRODUCTION

Over 100 X-linked conditions have been described in which mental retardation is a defining characteristic, usually in affected males, thus satisfactorily explaining the excess of males found in mentally retarded populations [Schwartz, 1993; Neri et al., 1994]. However, the frequency among females of carriers for such disorders is even greater, approaching 1 in 300 [Glass, 1991], and a significant proportion of these females demonstrate at least mild mental retardation. This latter observation derives, at least in part, from the confounding effect of X chromosome inactivation on the expression of X-linked genes in females. Thus, X inactivation has great significance for the study of X-linked mental retardation, from both a diagnostic and mechanistic standpoint.

X INACTIVATION AND THE X INACTIVATION CENTER

In somatic cells of normal females, one X chromosome is inactivated during early embryogenesis [Lyon, 1961; Willard, 1995]. X inactivation results in dosage compensation for most (but not all) X-linked genes, so that males and females express approximately equal amounts of products from such genes. X inactivation in early development is generally believed to be a random process, and females are accordingly mosaic for two populations of cells, expressing alleles from one or the other X chromosome [Davidson et al., 1963].

The process of X chromosome inactivation requires the presence, in *cis*, of a region of the proximal X chromosome long arm called the X inactivation center (XIC), which has been defined cytogenetically [Mattei et al., 1981; Therman et al., 1979] and molecularly [Brown et al., 1991a; Willard et al., 1993]. The principle evidence for such a locus derives from the finding that, if an X chromosome is involved in a balanced translocation to another chromosome, only one of the two products can undergo inactivation [Therman and Patau, 1974]. Consideration of inactive, structurally abnormal X chromosomes has allowed mapping of the XIC to a <1 megabase interval in Xq13.2. There is no convincing or substantiated evidence for more than a single XIC, and no chromosome lacking the XIC has been shown definitively to undergo X inactivation [Willard, 1995].

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Address reprint requests to H. F. Willard, Department of Genetics, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106.

Within the region currently defined as the XIC [Leppig et al. 1993; Brown et al., 1991a], the only gene known to date is the XIST gene, characterized by its exclusive expression from the inactive X. This finding, in addition to studies of XIST expression during early mouse development, implicates this gene in the initiation of the X inactivation process [Brown et al., 1991b; Kay et al., 1993]. The XIST gene and its RNA product has become an important molecular marker for the presence of an inactive X chromosome in clinical material.

There are two principle mechanisms whereby X chromosome inactivation has been shown to be involved in the expression of a mental retardation phenotype in females. First, non-random X inactivation has been documented in a number of cases of mentally retarded females who are either carriers of single-gene defects known to lead to mental retardation in hemizygous affected males or carriers of X chromosome structural abnormalities. Second, functional disomy (i.e., expression of both copies of an X-linked gene) has been implicated in cases of mental retardation, reflecting either failure of X inactivation due to physical separation of X-linked genes from the XIC or escape from X inactivation for a proportion of X-linked genes that are normally expressed from both active and (otherwise) inactive X chromosomes.

NON-RANDOM X INACTIVATION AND MENTAL RETARDATION

While the initial choice of which X is to be the inactive X in any cell is thought to be random, there are several instances in chromosomally normal females in which X inactivation appears to be non-random, that is, one or more tissues of an individual consist largely (if not exclusively) of cells expressing alleles from the same X chromosome, rather than being a mosaic of cells expressing alleles from one or the other X. While this situation in some cases reflects clear instances of post-inactivation cell selection in favor of or against a particular cell type [e.g., in X-linked immunodeficiencies or in X-linked alpha thalassemia with mental retardation; Gibbons et al., 1992], in many cases the apparent non-random inactivation presumably reflects the stochastic variation expected for a random event occurring in a relatively small number of embryonic cells at the time of X inactivation [Willard, 1995]. Given the small number of progenitor cells for any given tissue, one predicts on the basis of the binomial distribution that a significant number of females would show substantially skewed proportions of the two cell types purely by chance. Indeed, significantly skewed patterns of inactivation (e.g., 70:30 or 80:20 proportions of the two cell populations, instead of ~50:50) have been described in ~10–20% of females in surveys of normal females in the population [Gale et al., 1994; Naumova et al., 1996].

Molecular Methods

A number of useful molecular assays have been developed to examine the extent of random or non-random X inactivation in various tissues from heterozygous females. Many such assays are based on the principle of differential DNA methylation between the active and inactive X chromosome [Vogelstein et al., 1987; Hendriks et al., 1992; Allen et al., 1992; Maestrini et al., 1992; Carrel and Willard, this issue]. These assays examine the methylation patterns of restriction enzyme sites that correlate with the activity of the chromosome, in the vicinity of a polymorphism to distinguish the paternally or maternally derived copies of the gene or sequence in question [Vogelstein et al., 1987]. Digestion of genomic DNA with a methylation-sensitive enzyme cuts sites on the unmethylated active X, but not on the methylated inactive X. Subsequent Southern blotting or PCR, using primers that encompass the restriction sites as well as the polymorphism, is then used to determine what proportion of cells in a sample have one or the other allele on the active or inactive X.

An alternative and more direct approach is to examine the expression of polymorphic alleles that lie within transcribed regions of the X chromosome. One recently developed assay examined a transcribed polymorphism within the XIST gene [Rupert et al., 1995]. This site is heterozygous in about 50% of females, and the assay has been used to study several instances of non-random X inactivation in both chromosomally normal and abnormal females.

Clinical Consequences

Using such assays, the relevance of patterns of random or non-random X inactivation to the phenotypic expression in females of X-linked traits generally [e.g., Duchenne muscular dystrophy; Azofeifa et al., 1995; Pegoraro et al., 1995] and of X-linked mental retardation specifically has been amply demonstrated in studies that correlate the extent of skewed X inactivation with clinical severity. Among disorders characterized by mild or severe mental retardation in affected females, a clear correlation between X inactivation and severity has been documented for fragile X syndrome [Rousseau et al., 1991] and for pyruvate dehydrogenase deficiency [Brown et al., 1994; Dahl, 1995]. These correlations notwithstanding, it is important to emphasize that the X inactivation assays are usually performed on tissues (fibroblasts or lymphocytes) that are not obviously relevant to mental retardation. Thus, while the posited role of X inactivation in determining the clinical phenotype of heterozygous females is an attractive one, more direct studies, perhaps involving animal models, will be necessary to explore this relationship in detail for defects involving the brain.

The above correlations and the range of phenotypic expression observed for carriers of X-linked disorders are expected consequences of random X inactivation and variation in the proportion of cells with one or the other X active. Affected females with a high proportion of cells with the active X carrying a mutant allele are widely assumed to represent one extreme of a continuous distribution in the population. However, in an increasing number of instances, skewed X inactivation is apparent as a familial trait, that is, there are multiple affected females within the same family, each with the same degree of skewed X inactivation [e.g., Marcus

et al., 1992; Taylor et al., 1991]. Since the existence of such families is statistically improbable by chance alone, it is possible that they reflect inheritance of a genetic factor that controls the randomness or non-randomness of X inactivation. Alternatively, they may reflect the segregation of a defect in an X-linked gene influencing cell survival generally, as in the case of the XNP gene defect in X-linked alpha thalassemia with mental retardation [Gibbons et al., 1992; Stayton et al., 1994; Gibbons et al., 1995]. It may be productive to ascertain families with multiple mentally retarded females, as a possible source of examples of familial non-random X inactivation.

The clinical relevance of skewed X inactivation has also been suggested in the case of some females carrying deletions of the X chromosome. Typically, X chromosomes with large deletions have been found to be inactive in all cells, reflecting post-inactivation selection against any cells in which the deleted X is active (i.e., cells that are functionally nullisomic for the large number of genes missing from the deleted X); such is the case in cases of Ullrich-Turner syndrome with a 46,X,del(X) karyotype, for example. Presumably because the normal X is the active one in all cells, the clinical phenotype is relatively mild (equivalent to 45,X Turner syndrome cases) and mental retardation is not a feature.

In the case of smaller deletions, however, the situation is more variable. In these cases, one would predict that the extent of skewing might be less extreme, because selection against cells in which the deleted X is active is not as strong as it is when a substantial deletion is present. Thus, even for females in a family carrying the same deleted X, one finds a range of random and non-random inactivation patterns, just as one sees for normal X chromosomes. Several deletions involving Xq27 and Xq28 illustrate the consequences of X inactivation. Those with skewed inactivation of the normal X (i.e., those with a significant proportion of cells with the deleted X active) show features of mental retardation [Dahl et al., 1995; Clarke et al., 1992; Schmidt et al., 1990; Wolff et al., 1995]. In favorable families, the contrast between different female carriers in the same family has been quite illustrative of the importance of X inactivation patterns; females with the deleted X inactive in most cells were intellectually normal [Wolff et al., 1995].

FUNCTIONAL DISOMY AND MENTAL RETARDATION

It has been well established that all diploid somatic cells in both males and females have a single active X chromosome, regardless of the total number of X's or Y's present. While this observation has been central to models describing the initiation of X inactivation, it creates a paradox. If all X chromosomes in excess of one are inactive, as clearly demonstrated cytogenetically, then why are abnormal sex chromosome constitutions associated with clinical abnormalities? For example, the tetrasomy X syndrome (48,XXXX) is associated with significant mental retardation, and the pentasomy X syndrome (49,XXXXX) leads to severe mental and developmental retardation [Willard, 1995].

While there are a number of potential explanations, the fact that the clinical severity of X chromosome aneuploidy correlates with the number of extra inactive X chromosomes implicates a growing number of genes that have been shown to be expressed from both active and inactive X chromosomes [Disteche, 1995]. Such genes are said to "escape" X inactivation and are functionally disomic in normal females, in contrast to most X-linked genes that are functionally monosomic as a result of X inactivation. Recent studies have indicated that these genes are not rare and are located in many regions of the X chromosome, although there is mounting evidence that some regions (especially Xp22.3 and Xp11.2) contain a significantly higher number of genes that are expressed from the inactive X [Willard et al., 1993; Miller et al., 1995]. Thus, clinical abnormalities in cases of X aneuploidy may reflect the gene dose-dependent overexpression of dozens or perhaps hundreds of X-linked genes that do not undergo X inactivation (i.e., resulting in functional trisomy, functional tetrasomy, etc.). Viewed from this perspective, the pathogenesis of clinical abnormalities (including mental retardation) in these cases may not be different from that seen in autosomal trisomies [Epstein, 1986].

The above discussion dealt with X-linked genes that are normally expressed from both active and (otherwise) inactive X chromosomes. However, there are also circumstances in which genes that normally undergo X inactivation fail to do so because of a chromosome abnormality; in such cases, the resulting abnormal functional disomy can lead to severe clinical defects, including mental retardation. Partial functional disomies of the X chromosome are tolerated and are not lethal when the size of the noninactivated region is small [Schmidt et al., 1991; Du Sart et al., 1992; Schmidt and Du Sart, 1992].

Several cases have involved phenotypically abnormal girls with two structurally normal X chromosomes and an unbalanced X;autosome translocation involving Xp. Partial trisomy for the X chromosome segment was hypothesized to produce functional disomy in such patients due to the inability of genes in that segment to undergo X inactivation. For example, we reported a patient with an additional segment of Xp21.2→pter joined to the proximal short arm of chromosome 13 [Gustashaw et al., 1994]. Since the loss of most of the short arm of an acrocentric chromosome does not cause clinical abnormalities, the patient's phenotype could be attributed solely to the additional X chromosome material which appeared to escape X chromosome inactivation because the der(13) does not contain a copy of the XIC. Therefore, the additional segment would be expected to produce functional trisomy for those genes in Xp21.2→pter that normally escape X inactivation, and functional disomy for those genes that normally are subject to X inactivation in XX females. Similar reasoning has been applied to a case with Xp21.3→pter translocated to chromosome 21 [Ishikiriyama et al., 1993]. That patient's moderate mental retardation was attributed to functional disomy for genes in distal Xp that were inappropriately expressed in two doses due to the absence of X inactivation.

Small Marker or Ring X Chromosomes

A second class of patients with an abnormal phenotype and/or mental retardation due to functional disomy includes individuals with small ring or marker X [mar(X)] chromosomes in addition to a normal X. Such individuals often have mental retardation and dysmorphic features uncharacteristic of the phenotype associated with Ullrich-Turner syndrome [Kushnick et al., 1987; Van Dyke et al., 1992; Grompe et al., 1992; Dennis et al., 1993]. It has been hypothesized that the abnormalities seen in these patients may be due to a failure of X chromosome inactivation and the resultant functional disomy of pericentromeric sequences [Cohen et al., 1967; Van Dyke et al., 1991]. Recent molecular studies have confirmed this hypothesis, revealing that most small mar(X) chromosomes do not include the XIC and therefore cannot be subject to X inactivation [Migeon et al., 1993, 1994; Wolff et al., 1994]. Interestingly, the degree of clinical severity correlates with the size and/or the frequency of the mar(X)s lacking the XIC [Wolff et al., 1994], suggesting that functional disomy for specific genes in the pericentromeric region may account for the observed phenotypes. Patients with these large mar(X) chromosomes generally present only with symptoms of Turner syndrome, i.e., without mental retardation. Importantly, these chromosomes do contain the XIC [Wolff et al., 1994] and likely, therefore, undergo X inactivation normally. This correlation supports the mechanism of functional disomy in determining the severe phenotype associated with small mar(X) chromosomes [Cohen et al., 1967].

While the correlation between phenotypic severity and the absence of the XIC is convincing, it is not complete. Several female patients have been described who retain and express the XIST gene on the mar(X) and demonstrate a severe phenotype [e.g., Dennis et al., 1993]. While more comprehensive molecular analysis is required to fully understand these exceptions, one possibility may be that X inactivation of the mar(X)'s is either incomplete or abnormal in some respect, such that genes on the mar(X) are functionally disomic in at least a proportion of cells, despite expression of XIST. These exceptions notwithstanding, it is important to emphasize that the overwhelming majority of cases now reported support the relationship between a severe phenotype and absence of the XIC.

Gene Dosage and Phenotype

Figure 1 summarizes the clinical consequences of X chromosome inactivation as a determinant of levels of gene expression from X-linked genes. Under this simple gene dosage model, genes that are normally subject

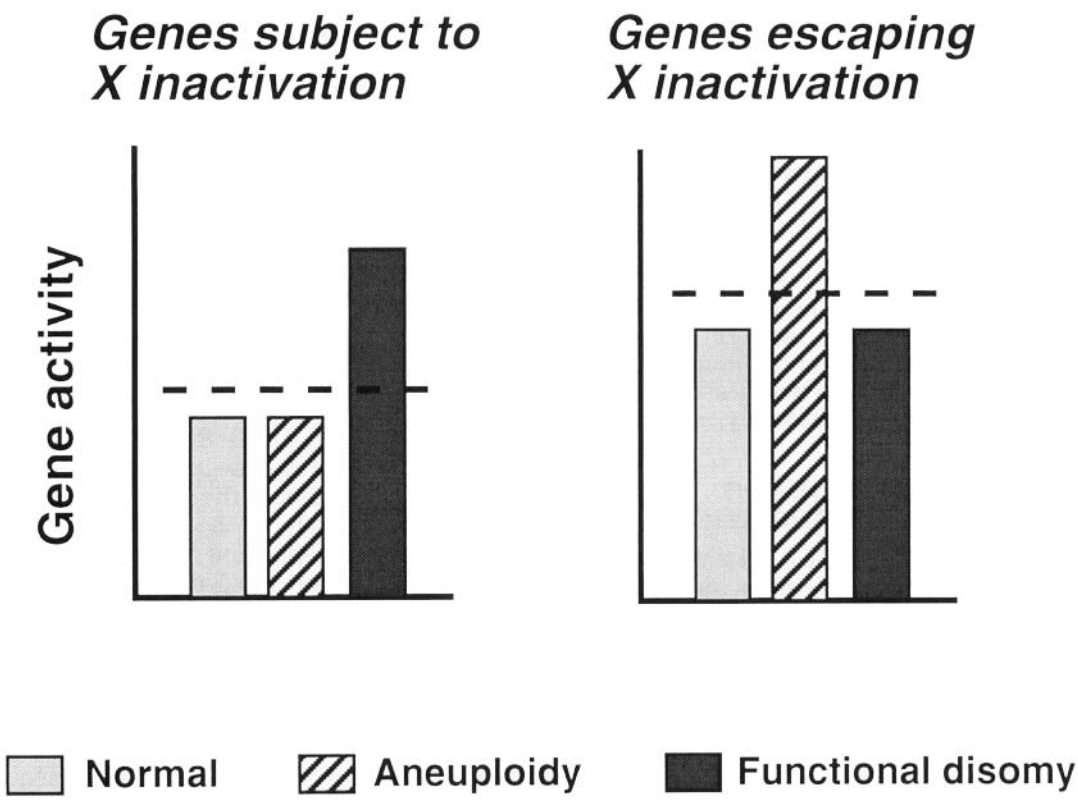

Fig. 1. Gene dosage models to explain the role of X-linked gene expression in determining the pathogenesis of X-linked disorders. Genes that are normally subject to X inactivation (left) or escape X inactivation (right) are implicated differently in cases of X chromosome aneuploidy or functional disomy (e.g., small mar(X)s). See text for discussion.

to X inactivation are dosage compensated and do not play a major role in the pathogenesis of abnormalities seen in individuals with X chromosome aneuploidy. When such genes are functionally disomic, however, as in the cases summarized above, they can lead to clinical abnormalities if their abnormally elevated levels of expression, secondary to the failure to undergo X inactivation, exceed a threshold consistent with normal development (Fig. 1, left). In contrast, the expression of genes that normally escape X inactivation would be predicted to be strictly dose dependent. Thus, in cases of aneuploidy, their overexpression might exceed the threshold, leading to abnormal mental and/or physical development (Fig. 1, right). One prediction of this gene dosage model would be that the severity of phenotype due to overexpression of these genes would increase proportionally to the number of extra X chromosomes; indeed, this appears to be the case if one compares the clinical phenotypes observed in XX, XXX, XXXX, and XXXXX females. In contrast to the situation with those genes that are subject to inactivation, genes that are normally functionally disomic in XX females (due to their escape from inactivation) would not be predicted to contribute to the abnormalities seen in patients with small centric mar(X) chromosomes, since their levels of expression would be the same in XX and X,mar(X) individuals irrespective of X inactivation.

SUMMARY

The role of X chromosome inactivation in influencing the expression of X-linked genes in females is especially significant in the case of X-linked mental retardation because of the large number of X-linked genes potentially involved [Neri et al., 1994]. There is now strong evidence that the penetrance of mental retardation in carrier females may be related to the randomness or skewing of X inactivation in critical tissues, whether determined stochastically or genetically. In addition, despite X inactivation, gene dosage considerations as a model to explain the pathogenesis of chromosome defects are as relevant for X-linked genes as they are for autosomal aneuploidies [Epstein, 1986], because of the fraction of X-linked genes that escape X inactivation. In either case, however, we lack definitive systems to evaluate the consequences of X inactivation or gene dosage in the brain where such effects are presumably most relevant. As genes involved in X-linked mental retardation are identified, they will need to be evaluated with respect to X inactivation to determine the extent to which the various mechanisms of genetic imbalance proposed here apply.

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